**Tautomerism in 8-Nitroguanosine Studied by NMR and Theoretical Calculations**

Thaís M. Barbosa, Roberto Rittner, Katie Alexander, Richard Cosstick, and Raymond J. Abraham

**ABSTRACT**

The guanine base in DNA, due to its low oxidation potential, is particularly sensitive to chemical modifications. A large number of guanine lesions have been characterized and studied in some detail due to their relationship with tissue inflammations. Nevertheless, one example of these lesions is the formation of 8-nitroguanosine, but the NMR data of this compound was only partially interpreted. A comprehensive study of the two possible tautomeric forms, through a detailed characterization of this compound, has implications for its base pairing properties. The target compound was obtained through a synthetic sequence of five steps, where all intermediates were fully characterized using spectral data. The analysis of the two tautomers was then evaluated through NMR spectroscopy and theoretical calculations of the chemical shifts and NH coupling constants, which were also compared with the data from guanosine.

**1. Introduction**

In 1863 Virchow noted a presence of leucocytes in neoplastic tissues and suggested a connection between inflammatory process and cancer. Even after all these years the understanding of the inflammatory microenvironment of malignant tissues has supported Virchow’s hypothesis, and the connection found between cancer and inflammation is starting to have implications for prevention and treatment.[1]

Epidemiological studies have shown that chronic inflammation inclines individuals to various types of cancer.[2] This is explained by the fact that inflammatory cells produce reactive oxygen and nitrogen species that have been proposed to induce DNA and tissue damage, which contribute to the multistage process of carcinogenesis.[3] Some examples of cancer that are based on inflammatory process are: liver cancer, which usually is a consequence of inflammation caused by chronic viral hepatitis; lung cancer, which results from inflammation caused by years of inhaled...
tobacco smoke, and the association between inflammatory stomach infections (such as *Helicobacter pylori*) and gastric cancer.\cite{1,4,5} It is estimated that underlying infections and inflammatory responses are linked to around 20% of all deaths from cancer worldwide.\cite{1,2}

One of the reactive nitrogen species (RNS) that can be formed in inflammatory cells is NO which reacts rapidly with superoxide anion (O$_2^\cdot$) to form peroxynitrite, which is a strong oxidant and can initiate reactions characteristic of hydroxyl radical (HO$^\cdot$), nitronium ion (NO$_2^+$) and nitrogen dioxide radical (NO$_2$)$^\cdot$.\cite{3} Previous studies of Yermilov and co-workers showed that guanine reacts rapidly with peroxynitrite under physiological conditions to form several substances.\cite{6} On the basis of chromatographic and spectral evidence, 8-nitroguanine was observed to be the major product from the reaction of peroxynitrite with guanine.\cite{6}

The guanine base in DNA, due to its low oxidation potential, is particularly sensitive, and a large number of guanine lesions have been characterized and studied in some detail.\cite{7,8} One example is the 8-nitro-2$'$-deoxyguanosine, which is know to labilize the glycosidic bond causing the release of 8-nitroguanine, which can be detected in urine, and leaves an abasic site in DNA (Figure 1).\cite{4,6,9}

The synthesis and study of oligonucleotides containing site-specific DNA lesions lead to an understanding of how chemical changes of DNA generate specific mutations. However, the high sensitivity of the 8-nitro-2$'$-deoxyguanosine glycosidic bond explains why the synthesis of oligodeoxynucleotides (ODNs) containing 8-nitroguanine by a conventional chemical approach has not been achieved. There is, however, strong evidence to suggest that this lesion is highly mutagenic and thus deserves a detailed study of its base pairing and other physicochemical properties.\cite{4}

To obtain a better understanding of the mutagenic effect of 8-nitro-2$'$-deoxyguanosine, Cosstick and co-workers described the synthesis of ODNs containing 8-nitro-2$'$-O-methylguanosine, in which the glycosidic bond is stabilized by a ribose sugar (Figure 2).\cite{4,10}

The detailed characterization of these C8-NO$_2$ lesions allowed the understanding of their biological consequences, particularly with regard to base pairing preferences and recognition by repair enzymes.\cite{11} There are at least two possible tautomeric forms (Figure 3) for the 8-NO$_2$-guanosine, both have implications in base pairing properties.

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**Figure 1.** Nitration of the guanine base in DNA by reactive nitrogen species and release of 8-nitroguanine to leave an abasic site in DNA.\cite{4}
This class of compounds which has a bulky substituent at the C8 position is reported to exist in the syn conformation about the glycosyl bond, as steric hindrance disfavours the anti-conformation; the syn conformation is known to be also promoted by intramolecular hydrogen bonding and other intermolecular features.\textsuperscript{[4,12]}

Two model molecules were reported to evaluate the keto-enol equilibrium in guanosine (Figure 4).\textsuperscript{[11]} The methyl group in 1-methyl-guanosine prevents C6 enolization, and the N1 atom has a chemical shift of 143 ppm. On the other hand, the O\textsuperscript{6}-alkylated guanosine is a model for the 6-enol-tautomer, and in this case N1 has a chemical shift of 214.1 ppm.

2. Experimental section

2.1 Flash column chromatography

The required quantity of silica (particle size 40–63 μM, supplied by BDH) was made into homogenous slurry with the column eluent and applied to the column over a base layer of sand and some cotton wool. The material to be purified was then introduced onto the column in the minimum volume of eluent or adsorbed into

![Diagram](image-url)
silica and then eluted with the eluent of choice. All fractions were collected and analysed by TLC.

2.2 Nuclear magnetic resonance spectroscopy (NMR)

All NMR spectra were recorded on a Bruker DPX 400MHz and Bruker Avance III 500 MHz; $^1$H NMR at 400 MHz or 500 MHz, $^{13}$C at 100 or 125 MHz and $^{15}$N at 50 MHz. All chemical shifts are reported in ppm and coupling constants (J) in hertz. $^1$H and $^{13}$C were referenced to the DMSO signal (2.54 ppm for $^1$H and 39.51 ppm for $^{13}$C), whereas $^{15}$N was referenced to the TMS signal.

2.3 High resolution mass spectroscopy (HRMS)

The mass spectrum was recorded using a Micromass LCT mass spectrometer. The sample was diluted in CH$_3$OH and analysed by negative mode electrospray ionization (ES$^-$) with a direct infusion syringe pump.

2.4 pH measurements

pH measurements were carried out using a Metrohm 691 pH meter. This was calibrated prior to use with buffers purchased from Thermo Russell (pH 4.00, 7.00 and 10.00 ± 0.01 at 25°C).

2.5 Reverse-phase high pressure liquid chromatography (RP-HPLC)

Reverse-phase HPLC was performed on an automated Gilson HPLC system equipped with an autoinjector, a photodiode array detector and a dual hydraulic
pump. Chromatographic data was manipulated using UniPoint Version 3.0 and absorbances measured at 254 nm.

**HPLC solvents:**
- **Buffer A:** (0.1 M aqueous TEAB solution, 500 mL) was prepared by diluting 50 mL of 1M TEAB solution with 450 mL of distilled water.
- **Buffer B:** (0.1 M aqueous TEAB solution, 500 mL) was prepared by diluting 50 mL of 1M TEAB solution with 250 mL of water and 200 mL of HPLC grade acetonitrile.

**HPLC control method:**
The eluent was gradually changed from 0% buffer B (100% buffer A) to 60% buffer B (40% buffer A) over 14 min, then gradually changed to 100% B over 2 min and kept 100% buffer B for another 2 min. After this, the eluent was gradually changed to 100% A over 2 min and kept 100% buffer A until the end of the run, which took a total of 25 min.

### 2.6 Synthetic steps to obtain 8-nitro-guanosine[4]

**Syntheses of 2′,3′,5′-tri-O-acetyl-guanosine (1)**

To a stirred suspension of guanosine (5.0 g, 17.7 mmol) in MeCN (65 mL) was added Et$_3$N (6.47 mL, 46.5 mmol) and DMAP (0.22 g, 1.8 mmol). The resulting suspension was cooled in an ice bath prior to dropwise addition of Ac$_2$O (5.84 mL, 61.84 mmol). Once the addition was completed, the mixture was allowed to reach room temperature and was stirred for a further 2 h. The reaction was quenched by the addition of MeOH (6.5 mL) and concentrated in vacuo to a white semi-solid precipitate which was boiled with iPrOH and filtered, leaving the product as a white solid. (5.79 g, 80%).

1H NMR (500 MHz, DMSO-d$_6$): δ (ppm) 10.70 (1H, s, NH); 7.91 (1H, s, H8); 6.49 (2H, s, NH2); 5.98 (1H, d, $^3$J$_{H1′}$H2′ = 6.1 Hz, H1′); 5.79 (1H, t, $^3$J$_{H2′}$H3′ = 6.0 Hz, H2′); 5.50 (1H, dd, $^3$J$_{H3′}$H4′ = 4.2, H3′); 4.37 (1H, dd, $^3$J$_{H5′}$H4′ = 3.8 and $^2$J$_{H5′}$H5′ = 11.5 Hz, H5′); 4.31 (1H, m, H4′); 4.26 (1H, dd, $^3$J$_{H5′}$H4′ = 5.7 Hz, H5′); 2.11 [3H, s, (CH$_3$)3′]; 2.04 [3H, s, (CH$_3$)5′]; 2.03 [3H, s, (CH$_3$)2′].

13C NMR (125 MHz, DMSO-d$_6$): δ (ppm) 170.89 [(CO)5′]; 170.24 [(CO)3′]; 170.07 [(CO)2′]; 157.46 (C6); 154.75 (C2); 151.95 (C4); 136.45 (C8); 117.74 (C5); 85.35 (C1′); 80.40 (C4′); 72.93 (C2′); 71.18 (C3′); 63.92 (C5′); 21.34 [(CH$_3$)5′]; 21.20 [(CH$_3$)3′] and 21.01 [(CH$_3$)2′].

**Syntheses of 8-bromo-2′,3′,5′-tri-O-acetyl-guanosine (2)**

Compound 1 (5.77 mg, 14.1 mmol) was suspended in distilled water (34 mL). Saturated bromine water (ca. 20 mL) was added with vigorous stirring at room temperature at such a rate that the orange colour dissipated between additions. Once the orange color persisted, the mixture was allowed to stir for a further 30 min before filtering. The solution was filtered and the solid washed with ice cold 1PrOH leaving the product as a pale orange solid. (5.79 g, 84%).

1H NMR (500 MHz, DMSO-d$_6$): δ (ppm) 10.86 (1H, s, NH); 6.55 (2H, s, NH2); 6.03 (1H, dd, $^3$J$_{H2′}$H1′ = 4.5 and $^3$J$_{H2′}$H3′
Syntheses of N2-dimethoxytrityl 8-bromo-2',3',5'-tri-O-acetyl-guanosine (3)

Product 2 (5.79 mg, 11.85 mmol) was added in a 250 mL bottom round flask and co-evaporated to dryness with anhydrous pyridine before re-dissolving in anhydrous pyridine (36.7 mL). The resultant solution was stirred at room temperature under an atmosphere of N₂ and dimethoxytrityl chloride (DMTCl) (4.40 g, 13.0 mmol) was added in ca. 0.5 g portions over 5 min. The mixture was stirred at room temperature for a further 8 h before removing most of the pyridine under reduced pressure. The isolated solid was partitioned between DCM (Dichloromethane) (50 mL) and water (50 mL), the DCM layer was further washed with water (2 × 50 mL) and saturated aqueous NaHCO₃ (30 mL) before drying over Na₂SO₄, filtering and evaporating to a yellow solid. Purification by flash column chromatography (100% DCM – 2% MeOH/DCM) gave the product as a yellow solid (5.9 g, 63%). ^1H NMR (500 MHz, DMSO-d₆): δ (ppm) 10.86 [1H, s, NH (1)]; 7.63 [H, s, NH (2)]; 7.31–7.28 (2H, m, DMT); 7.22-7.19 (3H, m, DMT); 7.15–7.12 (4H, m, DMT); 6.87–6.83 (4H, m, DMT); 5.43 (1H, d, ^3J_H1′_H2′ = 5.8 Hz, H1′); 5.30 (1H, t, ^3J_H2′_H3′ = 5.8 Hz, H2′); 4.82 (1H, t, ^3J_H3′_H4′ = 5.8 Hz, H3′); 4.30-3.99 (2H, m, H4′ and H5′); 3.81 (1H, dd, ^3J_H2′_H3′ = 12.2 Hz, H2′); 3.72 [3H, s, (CH₃)₃]; 3.71 [3H, s, (CH₃)₃]; 2.06 [3H, s, (CH₃)₃]; 2.00 [3H, s, (CH₃)₅]; 1.92 [3H, s, (CH₃)₂]. ^13C NMR (125 MHz, DMSO-d₆): δ (ppm) 170.81 [(CO)₅′]; 170.03 [(CO)₃′]; 169.75 [(CO)₂′]; 158.75 (DMT); 158.71 (DMT); 156.03 (C6); 152.42 (C2); 151.91 (C4); 145.97 (DMT); 137.17 (DMT); 130.52 (DMT); 130.45 (DMT); 129.10 (DMT); 128.73 (DMT); 127.56 (DMT); 121.06 (C8); 118.61 (C5); 113.98 (DMT); 113.93 (DMT); 87.64 (C1′); 79.33 (C4′); 70.63 (DMT); 70.41 (C2′); 70.24 (C3′); 63.59 (C5′); 55.83 (DMT); 21.43 [(CH₃)₅]; 21.16 [(CH₃)₃] and 20.84 [(CH₃)₂].

Syntheses of N2-dimethoxytrityl 8-nitro-2',3',5'-tri-O-acetyl-guanosine (4)

Product 3 (1.0 g, 1.26 mmol) was solubilized in a mixture of DMF (100 mL) and 18-crown-6 (3.33 g, 12.60 mmol). KNO₂ (1.07 g, 12.6 mmol) was added and the resulting mixture was stirred at 100°C for about 8 h under an inert atmosphere. A small sample of the reaction mixture was partitioned between ethyl acetate and water and the resulting organic layer was analysed by TLC (100% ethyl acetate). This was performed until the detection of degradation product was observed. The reaction was allowed to cool to room temperature, the Dimethylformamide (DMF) was evaporated under reduced pressure and the resulting solid was extracted with water (25 mL) and ethyl acetate (25 mL) three times. The organic layer was then washed
with saturated solution of NaHCO₃, dried over Na₂SO₄, filtered and dried over reduce pressure. Purification by flash column chromatography (ethyl acetate/hexane (1:1) – ethyl acetate/hexane (8:2)) leads to isolation of product 4 as a yellow solid (0.1 g, 13%). ¹H NMR (500 MHz, DMSO-d₆): δ (ppm) 11.16 [1H, s, NH (1)]; 8.03 [H, s, NH (2)]; 7.34-7.31 (2H, m, DMT); 7.26-7.21 (3H, m, DMT); 7.14-7.12 (4H, m, DMT); 6.89-6.87 (4H, m, DMT); 5.56 (1H, d, ³JH'₁-H₂' = 4.8 Hz, H₁'); 5.46 (1H, dd, ³JH'₂-H₃' = 7.2 Hz, H₂'); 5.15 (1H, t, ³JH'₃-H₄' = 7.0 Hz, H₃'); 4.14 (1H, dd, ³JH'₅-H₄' = 3.6 and ³JH₅'₅'' = 12.1 Hz, H₅'); 3.98 (1H, td, ³JH₄'₅'' = 6.2 Hz, H₄'); 3.87 (1H, dd, ³JH₅'' = 3.6 and ³JH₅'' = 11.7, H₅''); 3.73 [3H, s, (CH₃)DMT]; 3.72 [3H, s, (CH₃)DMT]; 2.05 [3H, s, (CH₃)3]; 1.99 [3H, s, (CH₃)5]; 1.78 [3H, s, (CH₃)2]. ¹³C NMR (125 MHz, DMSO-d₆): δ (ppm) 170.67 [(CO)₅']; 169.93 [(CO)₃']; 169.64 [(CO)2']; 158.84 (DMT); 158.79 (DMT); 156.84 (C₆); 154.18 (C₂); 152.12 (C₄); 145.50 (DMT); 143.72 (C₈); 137.32 (DMT); 136.88 (DMT); 130.51 (DMT); 130.47 (DMT); 129.08 (DMT); 128.71 (DMT); 127.66(DMT); 116.09 (C₅); 114.02 (DMT); 113.96 (DMT); 88.09 (C₁'); 79.12 (C₄'); 71.75 (C₂'); 70.88 (DMT); 69.48 (C₃'); 62.68 (C₅'); 55.88 (CH₃ (DMT)); 21.19 [(CH₃)₅']; 21.01 [(CH₃)₃'] and 20.66 [(CH₃)₂'].

Syntheses of 8-nitro-guanosine (5)

Product 4 (350.0 mg, 0.46 mmol) was dissolved in CHCl₃ before the addition of a solution of TsOH (38.0 mg, 0.23 mmol) in MeOH (4.6 mL). After 30 min, the solution was neutralized with triethylamine (32.0 μL, 0.23 mmol) and evaporated to dryness. The resulting solid was washed with ether a couple of times and the solution was monitored with TLC in 100% ethyl acetate until the spot corresponding to DMT disappeared. Then, the residue was dissolved in 7M NH₃ in MeOH, in a sealed vessel and stirred at room temperature for 48 h. The reaction was monitored by TLC using DCM in 15% of methanol and 2 drops of triethylamine, when no more reactant protected with the acetyl groups could be detected, the solvent and NH₃ were removed in vacuo. The resulting product contained traces of acid, so it was washed with isopropanol, evaporated and purified by HPLC. It led to the isolation of product 5 (6.9 mg, 4.6%). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 11.18 (1H, s, NH); 7.11 (2H, s, NH₂); 6.29 (1H, d, ³JH'₁-H₂' = 5.2 Hz, H₁'); 5.41 (1H, s, OH); 5.07 (1H, s, OH); 4.95 (1H, t, ³JH₂'₃' = 5.3, H₂'); 4.87 (1H, s, OH); 4.24 (1H, t, ³JH₃'₄' = 5.0, H₃'); 3.88 (1H, q, ³JH₄'₅' = 4.7, H₄'); 3.70 (1H, dd, ³JH₅'₅'' = 11.7, H₅''); 3.56 (1H, dd, H₅'''); 13C NMR (125 MHz, DMSO-d₆): δ (ppm) 157.72 (C₆); 156.84 (C₆); 154.18 (C₂); 152.12 (C₄); 145.50 (DMT); 143.72 (C₈); 137.32 (DMT); 136.88 (DMT); 130.51 (DMT); 130.47 (DMT); 129.08 (DMT); 128.71 (DMT); 127.66(DMT); 116.09 (C₅); 114.02 (DMT); 113.96 (DMT); 88.09 (C₁'); 79.12 (C₄'); 71.75 (C₂'); 70.88 (DMT); 69.48 (C₃'); 62.68 (C₅'); 55.88 (CH₃ (DMT)); 21.19 [(CH₃)₅']; 21.01 [(CH₃)₃'] and 20.66 [(CH₃)₂'].

3. Results and discussion

The synthesis of the 8-nitroguanosine derivatives is shown in Figure 5. The tautomerism analysis of 8-NO₂-guanosine (see Figure 3 for tautomeric forms) was performed in relation to the guanosine data, since it is reported to exist in the 6-keto form.[11] The 6-keto-nitroguanosine form is 0.3 kcal mol⁻¹ more stable than the 6-enol-nitroguanosine. This study took into account experimental values of ¹⁵N and
Figure 5. Synthesis of 8-nitroguanosine and its derivatives using the procedures outlined in Ref. 4.

Table 1. Experimental chemical shifts and coupling constants related to $^{15}$N of the reagent, intermediates and final product, as well as, the theoretical chemical shift of N1 for both tautomeric forms.

<table>
<thead>
<tr>
<th>N1 Chemical shift (ppm)</th>
<th>Experimental</th>
<th>Theoretical 6-keto form</th>
<th>Theoretical 6-enol form</th>
<th>$^{1}J_{N-H}$ Coupling constant (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanosine</td>
<td>148.2$^{*}$</td>
<td>180.37</td>
<td>244.51</td>
<td>88.4$^{*}$</td>
</tr>
<tr>
<td>(1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(2)</td>
<td>148.0</td>
<td>-</td>
<td>-</td>
<td>88.9</td>
</tr>
<tr>
<td>(3)</td>
<td>150.2</td>
<td>-</td>
<td>-</td>
<td>89.4</td>
</tr>
<tr>
<td>(4)</td>
<td>150.3</td>
<td>-</td>
<td>-</td>
<td>88.1</td>
</tr>
<tr>
<td>(5)</td>
<td>-</td>
<td>179.40</td>
<td>251.89</td>
<td>-</td>
</tr>
</tbody>
</table>

$^{*}$Ref.[90]

$^{13}$C chemical shifts, as well as $^{15}$N coupling constants. In addition to that, theoretical calculations were performed to evaluate the $^{13}$C and $^{15}$N chemical shifts; the geometry of the compounds was optimized at B3LYP and MP2 level of theory and aug-cc-pvDZ basis set, and the chemical shift values were obtained using BLYP level of theory and IGLO III$^{[13]}$ basis set (Tables 1–3).

The $^{1}$H–$^{15}$N HSQC contour maps of all intermediates involved in the 8-NO$_2$-guanosine synthesis provided us directly with the $^{15}$N chemical shifts, and its slice gave us the $^{1}J_{N-H}$. Figure 6 shows an example of $^{1}$H–$^{15}$N HSQC for 4, however the broad signal ($\sim$400 Hz) of H-1 from compound 5 precluded its analysis.

The analyses of Table 1 shows that neither NH chemical shifts nor NH coupling constants change significantly in both experimental and theoretical approach, which

Table 2. Experimental chemical shifts and coupling constants related to $^{15}$N of protected guanosine and protected NO$_2$-guanosine.

<table>
<thead>
<tr>
<th>N1 Chemical shift (ppm)</th>
<th>$^{1}J_{N-H}$ Coupling constant (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanosine-OAc-DMT</td>
<td>149.4</td>
</tr>
<tr>
<td>Guanosine-NO$_2$-OAc-DMT</td>
<td>150.3</td>
</tr>
</tbody>
</table>
Table 3. Experimental and theoretical $^{13}$C chemical shifts (ppm) for guanosine and 8-NO$_2$-guanosine.

<table>
<thead>
<tr>
<th></th>
<th>Experimental</th>
<th>Theoretical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>guanosine</td>
<td>8-NO$_2$-guanosine</td>
</tr>
<tr>
<td>C6</td>
<td>157.85</td>
<td>157.72</td>
</tr>
<tr>
<td>C2</td>
<td>154.71</td>
<td>156.48</td>
</tr>
<tr>
<td>C4</td>
<td>152.36</td>
<td>153.48</td>
</tr>
<tr>
<td>C8</td>
<td>136.65</td>
<td>144.44</td>
</tr>
<tr>
<td>C5</td>
<td>117.67</td>
<td>116.13</td>
</tr>
</tbody>
</table>

implies that there is no change in the tautomerism equilibrium among these compounds. Thus, as it was not possible to analyse the 8-NO$_2$-guanosine, it was decided to synthesize the guanosine with the acetyl and DMT protecting groups and compare their data with the corresponding values for 8-NO$_2$-guanosine with the protecting groups to detect the NO$_2$ effect (Table 2). The synthetic route to obtain the guanosine with the protecting groups was the same as described for 8-NO$_2$-guanosine apart from that only steps 1 and 3 were performed.[4]

The values shown in Table 2 confirm that the presence of the NO$_2$ group does not affect the tautomeric equilibrium, since no change in chemical shift or in coupling constants can be observed comparing the two compounds. The final analyses of tautomerism of 8-NO$_2$-guanosine were performed comparing experimental and theoretical $^{13}$C chemical shifts (Table 3). The theoretical chemical shift was calculated by the following equation:[13]

$$\delta = \sigma_{st} - \sigma_i.$$ (1)

Figure 6. $^1$H–$^{15}$N HSQC contour map of guanosine-NO$_2$-OAc-DMT, 4 (product from step 4).
where $\delta$ is the chemical shift and $\sigma_{st}$ and $\sigma_i$ are the shielding constants of the reference (TMS = 176.96 ppm) and the nuclei, respectively, which were calculated at the same level of theory.

It is clear that the enol tautomer exhibits a larger C6 chemical shift in comparison with the keto form for both compounds. The $^{13}$C spectrum shows only one signal in the region of 158 ppm. Therefore, it is clear that there is only one tautomer present, and it is the keto form for the guanosine and for the NO$_2$-guanosine.

4. Conclusion

The $^{15}$N chemical shift and coupling constant data, obtained through $^1$H-$^{15}$N HSQC and $^1$H-$^{15}$N HMBC contour maps, show that any structural modification could be detected from the values obtained for guanosine and 8-nitro-guanosine. Thus, it can be assumed that the NO$_2$ does not affect the tautomeric equilibrium, and it is predominantly in the keto-form. This idea was enhanced by experimental and theoretical data of $^{13}$C chemical shifts, which also did not show any modification.

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